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Thyroid Hormone Action in Cell Culture: Demonstration of Nuclear Receptors in Intact Cells and Isolated Nuclei

(receptor/thyroxine/triiodothyronine)

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ABSTRACT **Triiodothyronine and thyroxine induce a 3-fold increase in the rate of growth of GH_1 cells in culture. To study further the action of these hormones, we examined the binding of [^{125}I]triiodothyronine and purified [^{125}I]thyroxine to cellular fractions after incubation with intact cells in serum-free medium. High-affinity, low-capacity binding sites for the hormones were demonstrated in nuclear but not in mitochondrial or cytosol fractions. Chromatographic analysis of the bound nuclear radioactivity from cells incubated with [^{125}I]thyroxine demonstrated 97% thyroxine, 1% iodide, and 1% triiodothyronine. Apparent equilibrium dissociation constants, determined by Scatchard analysis, were 29 pM for triiodothyronine and 260 pM for thyroxine. The maximal binding capacity was identical for both hormones, with about 5000 sites per cell nucleus. [^{125}I]Thyroxine binding was competitively inhibited by triiodothyronine. These data suggest that triiodothyronine and thyroxine interact with identical nuclear receptors, and that conversion of thyroxine to triiodothyronine may not be a prerequisite for biologic activity. Similar high-affinity, low-capacity nuclear binding sites were also demonstrated by incubation of [^{125}I]triidothyronine directly with isolated nuclei. Incubation of cells with increasing concentrations of nonradioactive triidothyronine results in a subsequent increase in binding when [^{125}I]triiodothyronine is then incubated directly with isolated nuclei. This result suggests that nuclear receptors are not fixed, but increase after exposure of intact cells to hormone. This increase in nuclear receptor content may result from the transfer of an unstable cytosol receptor to the nucleus.**

Thyroid hormones affect the growth, development, and metabolism of virtually all tissues of higher organisms (1). In spite of extensive *in vivo* studies, the mechanism of action of thyroxine (T4) and triiodothyronine (T3) has not been determined. In addition, recent studies have demonstrated that T4 is converted to T3 by deiodination *in vivo* (2, 3). These studies have revitalized the concept that T4 functions as a prohormone and all its biologic activity results from its conversion to T3 *in vivo* (4).

We recently demonstrated that T3 and T4 induce a maximal 3-fold increase in the rates of growth and glucose utilization of GH_1 cells (a rat pituitary tumor cell line) in culture (5). These effects are induced by concentrations of hormone at physiologic levels in humans and other species as well as concentrations observed in thyrotoxic humans. Early effects of T3 and T4 on increasing RNA polymerase activity, nucleoside transport, and glucose utilization can be detected within 2–3 hr after incubation of hormone with intact cells (6). We examined the binding of T3 and T4 to various subcellular components of GH_1 cells. Saturable binding sites with affinities for T3 and T4 that correlate well enough with biologic dose–response relationships to be putative "receptors" for these hormones were detected only in the nuclear fraction. The interaction of hormone with these "receptors" can be demonstrated by incubation either with intact cells or with isolated nuclei.

Abbreviations: T3, triiodothyronine; T4, thyroxine; STM buffer, 0.25 M sucrose–20 mM Tris·HCl–1.1 mM $MgCl_2$, at pH 7.85 at 25°.

MATERIALS AND METHODS

Cell Suspensions and Media. GH_1 cells were obtained from the American Type Culture Collection. Cell cultures are routinely grown in 95% air–5% CO_2 with Ham's F-10 medium supplemented to 15% with horse serum and to 2.5% with fetal-calf serum (Grand Island Biological Co., Grand Island, N.Y.). The hormone concentrations in these sera are physiologic and are about 2 nM for T3 and 100 nM for T4, as determined by gas–liquid chromatography and specific radioimmunoassay (5). For hormone-binding studies, the medium was replaced with hypothyroid medium (Ham's F-10 medium supplemented to 10% with hypothyroid calf serum obtained from a thyroidectomized calf, Rockland Farms, Pa.) and the cultures were incubated for 24–48 hr to deplete the cells of hormone. The hormone concentrations in the thyroidectomized calf serum are 0.3 nM for T3 and 3 nM for T4 (5, 7).

Binding Studies with Intact Cells. For binding studies, the cells were harvested in the late logarithmic phase of growth with a rubber policeman and centrifuged at 500 × *g* for 5 min. The cell pellet was washed three times with 10 ml of serum-free Ham's F-10 medium by repeated dispersion and centrifugation. The cells were then suspended in serum-free medium with various concentrations of [^{125}I]T3 or [^{125}I]T4 and incubated at 37° for the times indicated. All radioactive analysis was done with a gamma spectrometer. After incubation with hormone, the cell suspensions were centrifuged at 500 × *g* for 5 min and the medium supernatant was saved for determination of hormone concentration. All further procedures were done at 0°. The cell pellet was homogenized in 10 volumes of STM buffer (0.25 M sucrose–20 mM Tris·HCl-1.1 mM $MgCl_2$, at pH 7.85 at 25°) by 15 strokes at 5000 rpm with a motorized pestle (Tri R, New York). The homogenate was centrifuged at 800 × *g* for 10 min, and the resultant supernatant was then centrifuged at 16,000 × *g* to obtain crude "mitochondrial" and "cytosol" fractions. The homogenate pellet was used to prepare nuclei either by centrifugation at 40,000 × *g* through 10 ml of 2.2 M sucrose or by two successive suspensions and centrifugations in 10 ml of STM–Triton buffer (0.25 M sucrose–20 mM Tris·HCl–

1.1 mM $MgCl_2$–0.5% Triton X-100 at pH 7.85 at 25°) (8, 9). Triton X-100 was obtained from Packard. Although nuclei isolated by centrifugation through 2.2 M sucrose appeared to be generally free of cytoplasmic contamination as determined by staining and phase-contrast microscopy, these nuclei required one to two additional treatments with STM–Triton buffer to eliminate a significant amount of low-affinity binding (unpublished data). The nuclear bound radioactivity did not decrease further with additional treatment with STM–Triton buffer. Both procedures isolated nuclei with protein/DNA ratios of about 2 and RNA/DNA ratios of 0.22. Protein, DNA, and RNA were determined (10–12).

Nuclear binding was also demonstrated with small numbers of cells without use of a homogenization step. The cell pellet (one to three million cells) was suspended in 1.0 ml of TM–Triton buffer (50 mM Tris·HCl–1.1 mM $MgCl_2$—0.5% Triton X-100), incubated for 5 min at 0°, and then centrifuged at 1000 × *g* for 8 min. This procedure was repeated once. This method for determining nuclear binding gave results equivalent to the homogenizing procedures indicated above.

Purification of [^{125}I]T3 and [^{125}I]T4. [^{125}I]T3 (initial specific activity, 286 Ci/mmol) and [^{125}I]T4 (initial specific activity, 389 Ci/mmol) were obtained from Abbott Laboratories, North Chicago, Ill. The purity of these compounds was examined by elution chromatography with Sephadex G-25 (fine) (13). The columns were equilibrated with 0.015 N NaOH–0.5 M NaCl, and 0.3–0.5 ml of hormone was applied to the column and then eluted with 0.1 N NaOH–5 mM NaCl. Iodide eluted within one column volume, T3 eluted in the second column volume, and T4 eluted in the third column volume. [^{125}I]T3 contained 2.0% iodide and no T4; [^{125}I]T4 contained 2.0% iodide and 2.5% [^{125}I]T3.

[^{125}I]T4 was purified by two successive paper chromatographic procedures and used immediately after the last elution (14). This preparation of [^{125}I]T4 was examined by Sephadex G-25 chromatography and was contaminated with less than 0.8% [^{125}I]T3.

Analysis of Radioactivity Bound to Nuclei. To determine the nature of the nuclear bound radioactivity, nuclei were suspended in 0.015 N NaOH–0.5 M NaCl and applied directly to Sephadex G-25 fine columns. The columns were eluted as described above. This procedure extracted more than 98% of the bound radioactivity, and less than 2% was detected in the excluded volume of the column.

Demonstration of Nuclear Receptors by Direct Incubation with Isolated Nuclei. Nuclei were prepared by either of the homogenization procedures indicated above. When they were prepared by centrifugation through 10 ml of 2.2 M sucrose, the nuclei were washed twice in 10 ml of STM–Triton buffer before incubation to remove cytoplasmic contamination. The incubation mixture contained a nuclear suspension equivalent to 20–50 μg of DNA, 0.25 M sucrose, 20 mM Tris·HCl–1.1 mM $MgCl_2$ (pH 7.85 at 25°), and 2.0 mM, NaEDTA, in 0.9 ml. The incubation mixture was prepared at 0°. [^{125}I]T3 was added to the incubation mixture in 0.1 ml of serum-free Ham's F-10 medium. Hormone binding was studied by incubating the nuclear preparation at 37°. At the indicated times, the samples were chilled in an ice bath and then centrifuged at 1000 × *g* for 8 min. The nuclear pellet was suspended in 1 ml of STM–Triton buffer and centrifuged again. "Specifically" bound hormone in the nuclear pellet was considered to be that which was inhibited by a molar excess (100–200 fold) of nonradioactive hormone.

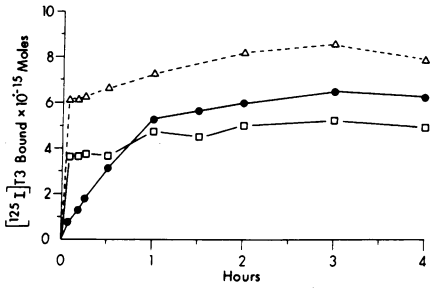

FIG. 1. Time course of binding of [^{125}I]T3 to subcellular components of GH_1 cells. Cells (1.25 × 10^6) were incubated in 1 ml of serum-free medium with 10 pM [^{125}I]T3 at 37°. At the times indicated they were chilled and fractionated to prepare "nuclei" (●), "mitochondria" (□), and "cytosol" (Δ). The results reflect hormone bound to the respective cell fraction isolated from 1.25 × 10^6 cells. 1.25 × 10^6 cells contain 15 μg of DNA, 20 μg of "mitochondrial" protein, and 80 μg of "cytosol" protein. Each time point represents the average of three determinations, which did not vary by more than 5%.

RESULTS

Binding of T3 to Subcellular Components in Intact Cells. Fig. 1 illustrates the time course of binding of T3 to nuclei, mitochondria, and cytosol isolated after incubation of [^{125}I]T3 with intact cells at 37°. Binding to mitochondria and cytosol occurs very rapidly and approaches an equilibrium within 5–10 min. Binding of T3 to nuclei occurs more slowly and attains a maximal value only after 2–3 hr of incubation. The kinetics of binding for mitochondria and cytosol at 0° is similar to that determined at 37°. Nuclear binding, however, is completely inhibited at 0° (unpublished data). The time course of T4 binding was similar to that of T3.

It is likely that cells contain multiple binding components of varying affinities for T3 and T4. High-affinity, low-capacity (saturable) binding sites with an equilibrium dissociation constant similar to the concentration of hormone inducing a half-maximal biologic effect would likely function as hormonal receptors. Low-affinity and high-capacity (nonsaturable) binding sites would likely represent a "nonspecific" interaction. To differentiate between "specific" and "nonspecific" binding to cell components, we calculated *R*, the ratio of moles of hormone bound to the hormone concentration at equilibrium. *R* is then plotted against the hormone concentration (15). Theoretically, for saturable binding sites, as the molar concentration of hormone approaches the dissociation constant for the binding protein, the value of *R* decreases, and as the bound hormone approaches a constant value at saturation, the value of *R* approaches 0. For high-capacity "nonspecific" binding sites the value of *R* remains constant with increasing hormone concentrations.

In a typical experiment (Fig. 2), over a T3 concentration range of 10^{-12}–10^{-8} M, the value of *R* remains constant for the mitochondrial and cytosol fractions. The nuclear fraction, however, demonstrates a marked decrease in the value of *R* up to 1 × 10^{-10} M. At higher T3 concentrations the value of *R* tends to level off at a value greater than 0, reflecting binding to high-capacity "nonspecific" sites.

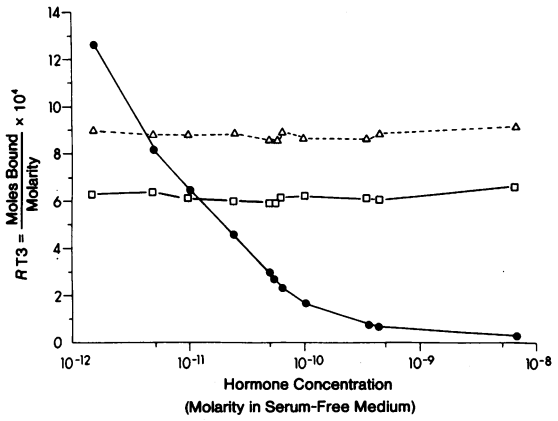

FIG. 2. Binding of [^{125}I]T3 to subcellular components of GH_1 cells at equilibrium. 3×10^6 GH_1 cells were incubated in 2 ml of serum-free medium with various concentrations of [^{125}I]T3 at 37° for $2^1/_2$ hr. The cells were separated into nuclear, mitochondrial, and cytosol fractions, and the medium was saved for determination of hormone concentration. R, the ratio of the moles of T3 bound to the T3 concentration at equilibrium was determined at each concentration for nuclei (●), mitochondria (□), and cytosol (△). 3×10^6 cells contain 35 μg of DNA, 50 μg of "mitochondrial" protein, and 195 μg of "cytosol" protein.

Binding of T4 and T3 to Nuclei. Fig. 3 illustrates the results of a combined experiment studying [^{125}I]T4 and [^{125}I]T3 binding to nuclei, after incubation with intact cells. The values of R for cytosol and mitochondria are not illustrated. These R values for T3 were similar to those determined in Fig. 2. The cytosol and mitochondrial R values for T4 remained constant over the T4 concentration range and were 80% of the value observed for T3. The values of R for T4 in the nuclear fraction are less than those for T3 and the plot of R against the T4 concentration is shifted to the right of the T3 binding curve. This result suggests that the nuclear "receptor" has a lower affinity for T4 than for T3. Fig. 4 illustrates an estimation of the maximal binding capacity and the apparent equilibrium dissociation constants for T3 and T4 determined by the Scatchard method (16). The apparent K_d for T3 is 29 pM and the apparent K_d for T4 is

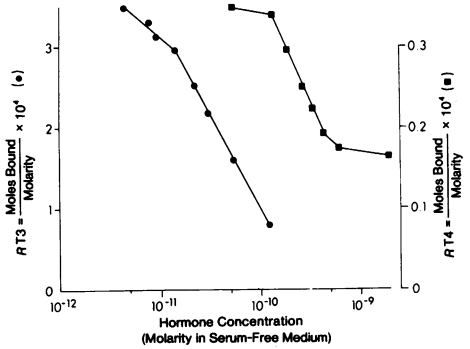

FIG. 3. Binding of [^{125}I]T3 and [^{125}I]T4 to nuclei. GH_1 cells (1.5×10^6) were incubated in 1 ml of serum-free medium with various concentrations of [^{125}I]T3 or [^{125}I]T4 at 37° for $2^1/_2$ hr. The nuclei were isolated and the medium was saved for determination of hormone concentration. The binding at each hormone concentration represents the average of three determinations, which did not vary by more than 5%. R is the ratio of moles of hormone bound per 20 μg of DNA to the hormone concentration at equilibrium.

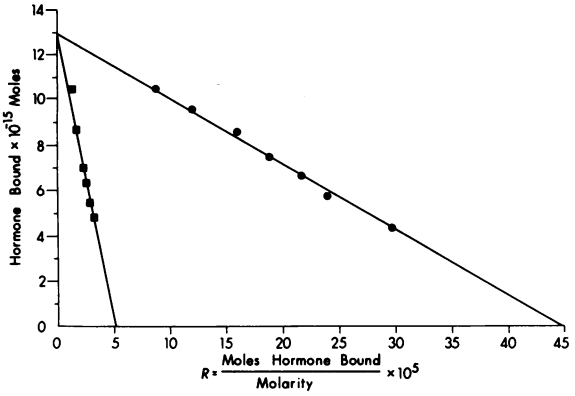

FIG. 4. Scatchard analysis of nuclear binding of T3 (●) and T4 (■). The experiment was the same as in Fig. 3. Note that the data for T4 was constructed with the six lowest T4 concentrations from Fig. 3. The values of R for the two highest T4 concentrations were omitted because about 50% of the T4 binding at these concentrations consists of nonspecific, low-affinity, high-capacity binding (Table 1). Although the extent of low-affinity binding is not as substantial at lower T4 concentrations, it might result in a slight underestimation of the K_d for T4. This is not a problem for a Scatchard analysis of T3 binding since low-affinity, high-capacity binding represents less than 5% of total T3 binding between concentrations of 5 and 100 pM.

260 pM. The maximal binding capacity is similar for both hormones, 13×10^{-15} moles bound per 20 μg of DNA. This represents about 5000 molecules of T3 or T4 bound per cell nucleus at saturation.

Alternatively, the apparent T4 binding may be T3 formed as a result of conversion of T4 to T3. We, therefore, examined the nuclear bound radioactivity from cells incubated with [^{125}I]T4 to exclude the possibility that the bound hormone was [^{125}I]T3 formed as a result of conversion of T4 to T3 during incubation. The results obtained by chromatography with Sephadex G-25 (fine) indicated that the bound radioactivity consisted of 97% [^{125}I]T4, 1% iodide, and 1% [^{125}I]-T3. The remaining 1% of the bound radioactivity eluted within the excluded volume of the column. A similar study with nuclei from cells incubated with [^{125}I]T3 demonstrated 98% T3 and 1% iodide. About 1% of the radioactivity also eluted in the excluded volume of the column. These results indicated that hormone bound to nuclei can be easily dissociated in unaltered form and that [^{125}I]T4 binding does not

TABLE 1. *Inhibition of [^{125}I]T4 binding by nonradioactive T3 and T4**

Hormone concentration (nM)	[^{125}I]T4 bound (fmol)
[^{125}I]T4 (1)	24
[^{125}I]T4 (1) + T4 (100)	12
[^{125}I]T4 (1) + T3 (100)	11

* The effect of a 100-fold molar excess of nonradioactive T4 and T3 was studied on the binding of [^{125}I]T4 to nuclei of GH_1 cells as part of the experiment indicated by Figs. 3 and 4 with identical conditions. The results are expressed as [^{125}I]T4 bound per 20 μg of DNA. The inability to completely inhibit [^{125}I]T4 binding by nonradioactive T3 or T4 reflects low-affinity, high-capacity binding and accounts for about 50% of the total [^{125}I]T4 bound at 1 nM (see Fig. 4).

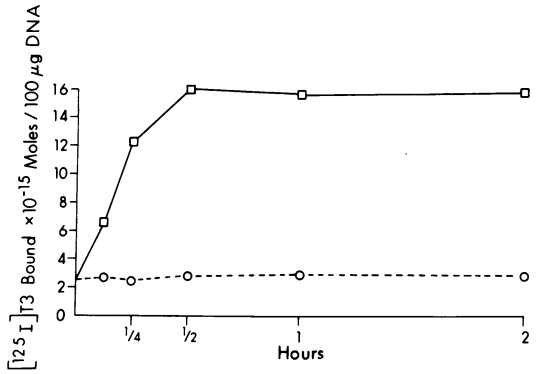

FIG. 5. Time course of binding of [125I]T3 to isolated nuclei. Nuclei isolated from GH_1 cells cultured in growth medium were incubated at 37° with 0.1 nM [125I]T3 with (○) and without (□) a 100-fold molar excess of nonradioactive T3. At the indicated times the nuclei were chilled, centrifuged, and washed once with STM–Triton buffer. Hormone binding at zero time for both concentrations of T3 was determined after a 1-min incubation of hormone and nuclei at 0°. Each point represents the average of three determinations, each of which did not vary from the average by more than 5%.

result from conversion of T4 to T3 during the incubation. If T3 and T4 interact with identical rather than different classes of receptors, binding of [125I]T4 to nuclei should be inhibited to a similar degree by a 100-fold molar excess of nonradioactive T3 or T4 (Table 1). The inhibition of [125I]T4 binding by a 100-fold molar excess of T3 or T4 is equivalent to the calculated "specifically" bound [125I]T4 determined from Fig. 4. The fact that the maximal binding capacity is identical for T4 and T3 and that T3 inhibits T4 nuclear binding suggests that both hormones interact with identical receptor sites in the nucleus.

The Relation Between T3 and T4 Nuclear Binding and the Biologic Effect in GH_1 Cells. Both T3 and T4 exist in bound and free forms in serum, with the free fraction correlating best with biologic activity. The estimated free hormone concentrations inducing a half-maximal increase in the rates of cell growth and glucose utilization are 8 pM for T3 and 100 pM for T4 (5). These values are slightly lower than the hormone concentrations giving half-maximal nuclear binding. This may be due to the fact that free hormone concentrations in the biologic studies are estimated by equilibrium dialysis (17). Therefore, estimated free hormone concentrations may not give an accurate determination of the value *in vivo*. Nevertheless, the biologic and nuclear binding data are similar enough to suggest that the high-affinity, low-capacity nuclear binding sites function as cellular receptors and in some way mediate the action of the thyroid hormones.

Demonstration of Nuclear Receptors by Direct Incubation with Isolated Nuclei. The kinetics of [125I]T3 binding to nuclei is illustrated in Fig. 5. Equilibrium with nuclear receptors is attained more rapidly with isolated nuclei than after incubation with intact cells. Nevertheless, the binding observed with isolated nuclei appears to be "specific", since binding of [125I]T3 is markedly inhibited by a 100- to 200-fold molar excess of nonradioactive hormone.

Variability in Nuclear Receptor Content. Glucocorticoid and estrogen in target tissues initially interact with a cytosol receptor (18, 19). This hormone–receptor complex then binds to specific acceptor sites in the nucleus. The time course of binding of thyroid hormone to cytosol and nuclei (Fig. 1) does not demonstrate a shift of possible cytosol "receptors" to the nucleus. In addition, specific, high-affinity, low-capacity binding sites were not detected in the cytoplasmic fraction but are demonstrated in the nuclear fraction (Fig. 2). This may result from the fact that a putative cytoplasmic receptor is labile, but when it is transferred to the nucleus as a hormone–receptor complex, it is stable. If the scheme of T3 binding to nuclei in intact cells is similar to that observed for steroids, the number of nuclear receptors is not fixed and should increase after incubation of intact cells with hormone (20). To test this possibility, we incubated intact cells with increasing concentrations of nonradioactive T3, and then examined the extent of [125I]T3 binding in a second incubation with isolated nuclei. If nuclear binding sites were fixed in number, the lower the concentration of T3 in the first incubation, the greater the extent of [125I]T3 binding present in the second incubation. However, if a putative cytoplasmic receptor were involved in T3 nuclear binding, preincubation of cells with nonradioactive T3 might increase the number of high-affinity nuclear binding sites for T3. This might result in an increase in [125I]T3 binding to isolated nuclei in the second incubation. Incubation of intact cells with increasing concentrations of nonradioactive T3 resulted in a subsequent increase in the magnitude of [125I]T3 binding to isolated nuclei (Fig. 6). This result suggests that the number of apparent nuclear receptor sites is not fixed but increases after incubation of intact cells with hormone, possibly from the transfer of an unstable cytoplasmic receptor to the nucleus.

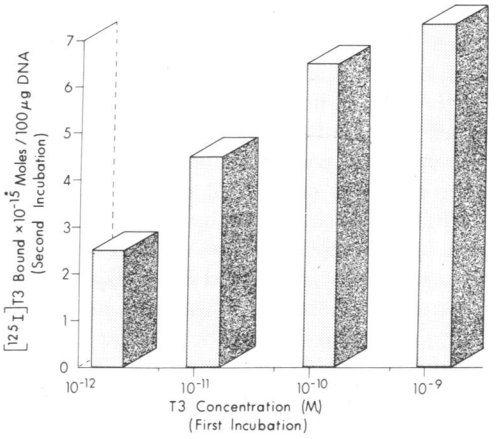

FIG. 6. Variability in nuclear receptor content. GH_1 cells (20×10^6) were incubated in 10 ml of serum-free medium at 37° for 1 1/2 hr with the four concentrations of nonradioactive T3 indicated. The nuclei were then isolated by the Triton X-100 method, and each group of nuclei from the first incubation was then incubated with 100 pM [125I]T3 with and without a 200-fold molar excess of nonradioactive T3. The moles of [125I]T3 bound from the second incubation reflects subtraction of low-affinity binding determined from the incubation with the 200-fold molar excess of nonradioactive T3. The extent of low-affinity binding was about 2 fmol of [125I]T3 per 100 μg of DNA and was independent of the concentration of T3 of the first incubation. Each point represents an average of three determinations, each of which did not vary by more than 10%.

DISCUSSION

These studies indicate that GH_1 cells contain high-affinity, low-capacity binding sites for both T3 and for T4 in the cell nucleus. The concentrations of T3 and T4 inducing a half-maximal biologic effect and half-maximal nuclear binding are sufficiently similar to suggest that these nuclear binding sites function as cellular receptors for these hormones. The fact that T4 is converted to T3 *in vivo* has suggested the possibility that all of the biologic activity of T4 results from its conversion to T3. The recent demonstration of apparent saturable nuclear binding sites for T3 but not for T4 in rat liver, pituitary, and kidney after administration of hormone to intact animals gives additional support to the concept that T4 lacks intrinsic biologic activity (4). The difficulty in easily detecting high-affinity binding sites for T4 in these *in vivo* studies may be related to the fact that T4 binding might be obscured by a significant degree of high-capacity, low-affinity binding sites present in nuclei isolated by centrifugation through 2.2 M sucrose. In GH_1 cells, high-affinity, low-capacity nuclear binding sites for T4 could not be easily demonstrated unless isolated nuclei were treated twice with buffer containing 0.5% Triton X-100. This procedure solubilizes the outer nuclear membrane as well as any cytoplasmic contamination that would obscure the detection of saturable binding sites for T4 (9).

"Specific" nuclear binding of T3 is diminished by addition of hormone-depleted serum to an incubation mixture containing either intact cells or isolated nuclei (unpublished data). This finding suggests that "specific" nuclear binding is proportional to the free rather than the bound fraction of hormone in serum. Based on the unbound hormone concentrations, our biologic and nuclear binding studies suggest that on an equimolar basis, T4 has about 1/10 the intrinsic biologic activity of T3. The physiologic concentrations of the thyroid hormones in serum are about 2 nM for T3 and 100 nM for T4, of which greater than 99% is in the bound form (17). The relative contribution of T3 and T4 to the total biologic activity of the thyroid hormones in serum is likely dependent on both their relative intrinsic biologic activities as well as on the free hormone concentrations present in serum. The precise concentrations of free T3 and T4 *in vivo* have not been determined. Estimations by equilibrium dialysis of the percent of total hormone free at physiologic concentrations are about 0.03% for T4 and 0.2–0.4% for T3 (17, 21). The calculated free hormone concentrations determined by multiplying the percent free by the total hormone concentration is about 6 pM for T3 and 30 pM for T4. On the basis of these concentrations, if T4 has 1/10 the intrinsic biologic activity of T3, it contributes to about 33% of the biologic activity of the thyroid hormones at physiologic concentrations.

Previous attempts to demonstrate binding of T3 or T4 by incubation directly with isolated nuclei have been unsuccessful (4, 22). These studies used incubation mixtures containing 3–10 mM Mg^{++}. Our studies suggest that the interaction of hormone with saturable binding sites in isolated nuclei is inhibited by divalent ions (unpublished data) and can be demonstrated after addition of chelating agents to the incubation mixture. It is not clear whether chelating agents increase the rate of dissociation of T3 bound to nuclear receptors, allowing an exchange reaction with [^{125}I]T3, or increase the permeability of the nuclear membrane to [^{125}I]T3. Recently, by extraction of nuclei with 0.4 M KCl, we solubilized a high-affinity T3 binding activity that appears to be an acidic nonhistone protein (unpublished data). The kinetics of binding of [^{125}I]T3 to this fraction is similar to that observed for isolated nuclei, and the binding is also enhanced by chelating agents. This finding suggests that Na EDTA increases the binding of T3 directly to nuclear receptors rather than alters nuclear membrane permeability.

Our studies in isolated nuclei suggest that the number of nuclear receptors for thyroid hormones is not fixed but increases after incubation of intact cells with T3. These results suggest that the characteristics of binding of the thyroid hormones to nuclear sites may occur by molecular mechanisms similar to that described for estrogens and glucocorticoids in target tissues.

The excellent correlation between the biologic dose–response relationship and hormone binding to nuclei suggests that these high-affinity nuclear "binding sites" play an important role in mediating the action of the thyroid hormones. The localization of the high-affinity binding activity to the cell nucleus supports the concept that the thyroid hormones modulate gene activity.

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